Discriminant analysis in allergic rhinitis and asthma: methacholine dose–response slope allows a good differentiation between mild asthma and rhinitis

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Abstract Asthma and rhinitis frequently coexist in allergic patients, but nasal symptoms may predominate, leading to asthma underdiagnosis and undertreatment. Discriminant analysis obtains the best differentiation between groups using one or one set of variables. Our aim was to identify the laboratory test [allergen exposure, total and specific serum IgE, lung function, blood eosinophils and, bronchial response and sensitivity to methacholine (Mth) and allergen] or combination of them that allowed the best differentiation between mild asthma and allergic rhinitis. A cross-sectional analysis was performed in 86 *Dermatophagoides pteronyssinus* allergic rhinitis patients, who were classified according to clinical data as rhinitis plus mild asthma ($n = 62$) or "pure" rhinitis ($n = 24$). Bronchial symptoms had been exhaustively evaluated during a 2-years pre-inclusion period. Patients underwent skin tests and bronchial challenge with Mth and allergen. The exposure to *D. pteronyssinus* allergen (Der pI) was quantified in dust samples. Dose–response curves with Mth [until the FEV_1 fell by 40% or the maximal dose (200 mg/ml) was inhaled] were attained. We developed multiple models of discriminant analysis in order to evaluate the capacity of the above variables to differentiate groups. Asthma patients had higher total and specific IgE levels and a greater sensitivity (PD_{20} values) and response [dose–response slope (DRS)] to both Mth and allergen. The model entering these variables was the one that correctly classified more patients (79.2%). The discriminative power of the model that only included Mth-DRS values was similar to the above (78.8%). Bronchial response to Mth is quantitatively different in allergic rhinitis patients who display mild asthma symptoms when compared to those that only report rhinitis, suggesting a distinct bronchial intrinsic behavior. The utilization of complete dose–response curves with Mth allows a good separation between mild asthma and "pure" rhinitis patients and might be useful in the diagnosis of mild asthma. Whether the early detection and treatment of these patients prevents the development of symptomatic asthma needs further evaluation.

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Keywords allergen; bronchial challenge; discriminant analysis; dose–response slope; methacholine; mild asthma, rhinitis.

INTRODUCTION

Asthma and rhinitis frequently coexist in atopic patients. Rhinitis often precedes the onset of bronchial symptoms and is considered an independent risk factor for asthma(1). Rhinitis patients' bronchial airways exhibit changes (eosinophilic infiltration and activation, subepithelial fibrosis and epithelial cell shedding) similar to those described in asthmatics (2–4). In rhinitis patients, natural (5) or experimental (6–8) exposure to the allergen enhances bronchial hyperresponsiveness (BHR) and eosinophilic inflammation, resembling the asthmatic response. Further, the isolated treatment of rhinitis indirectly improves asthma symptoms and decreases BHR (9). These data suggest a common pathophysiologic origin of both conditions and the lack of a clear differentiation between them. Factors determining that subjects develop asthma or only rhinitis are far from clear.

The benefit of early anti-inflammatory treatment in asthma has been documented (10). Due to the low sensitivities of both, lung-function and biochemical tests (11), the early detection of mild asthma patients is troublesome and often lies in clinical history (recurrent episodes of airway narrowing) (12). The highly variable individual perception of these episodes (13) can lead to a serious underdiagnosis of the disease (14). In some instances,

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the allergen-bronchial provocation test (A-BPT), a valuable model for the study of allergic asthma (AA) pathogenesis, has been considered the "gold standard" in the diagnosis of the disease (15). But rhinitis patients, who have never experienced asthma symptoms, can positively respond to A-BPT (6,7) which questions the capacity of such tests to differentiate both conditions.

We selected *Dermatophagoides pteronyssinus* monosensitized patients who, in order to be accurately classified in the groups of "rhinitis and mild asthma" and "pure rhinitis," had been evaluated during a pre-inclusion period of 3 years. Multiple models of discriminant analysis were developed in order to test the capacity of different asthma-associated variables [lung function, allergen exposure, eosinophils, total and specific IgE and, bronchial response to methacholine (Mth) and allergen] to separate patients displaying mild asthma from those only reporting upper airway symptoms.

PATIENTS AND METHODS

Patients and study design

We recruited 86 non-smoking *D. pteronyssinus*- monosensitized patients who had been evaluated for a 3-year period (median); interquartile range (IQR): 2–4 years. During this time, bronchial symptoms (recurrent attacks of wheezing, cough, breathlessness and chest tightness) (16) had been repeatedly interrogated. Patients were then classified as "mild asthma and rhinitis" ($n = 62$) and "pure rhinitis" ($n = 24$). Only short-acting β-agonist drugs used "as needed" were allowed in the previous 2 months. The selection of patients was not carried out according to the presence of BHR. No subject had suffered from lower or upper respiratory tract infections or had been treated with oral or inhaled corticosteroids within the preceding 2 months. *Dermatophagoides pteronyssinus* allergy was established by positive skin-prick tests and specific IgE to this allergen, in the context of a compatible clinical history. Patients sensitized to other allergens were excluded.

Once patients were grouped, the study was carried out over 2 consecutive days. First, patients underwent clinical and physical evaluation, venous blood was sampled, skin tests and the methacholine-bronchial provocation tests (Mth-BPTs) were done and, dust from patients' beds was collected. The A-BPT was performed the following day. Informed written consent was obtained from all patients before starting the study. The ethics committee of our hospital approved the study protocol.

Allergen extract

An extract of *D. pteronyssinus* partially purified and biologically standardized was used for skin and bronchial challenge tests (ALK-Abelló, Spain). Specific monoclonal antibodies were used to quantify the major mite allergens (Der p I and Der 2) (17). The final extract at 100 biologic units (BU)/ml contained 40 μg/ml of Der p I and 20 μg/ml of Der 2.

METHODS

Collection of dust samples and quantitation of mite allergens

Dust collection was carried out 7 days after the last cleaning, as previously described(18). A portable vacuum cleaner provided with a prefilter (ALK, Denmark) was used(19). After vacuuming, the filter box was removed and stored at −20°C until further analysis. The content of Der p I allergen was determined in duplicate by a commercial enzyme-linked immunosorbent assay (ELISA) based on monoclonal antibodies (ALK-Abelló. Denmark) (17).

Skin tests

Skin-prick tests were carried out with a standard battery that included the most common airborne allergens in our area (mites, pollens, molds, and animal dander) (ALK-Abelló, Spain) (20). We also tested the *D. pteronyssinus* extract (containing 50% glycerin, 0.4% phenol, and 0.9% NaCl) at three concentrations (60, 12, and 2.4 μg/ml of Der p I) in duplicate and in the inverse direction. The resulting wheals were shaped and transferred to a millimeter-squared paper, where areas were measured and expressed in millimeters squared. We considered for analysis the mean value of the skin areas obtained from the six tests.

Bronchial provocation tests

Every lung-function test was performed with the same spirometer (model CPR, Medical Graphics inc. St Paul, MN, U.S.A.). Spirometry (21) and BPTs (22) were made according to international guidelines. Reference values of Crapo *et al*. (23) were used. Mth or allergen extract was administered by a dosimeter (MEFAR s.r.l.; MEFAR, Borezzo, Italy), programmed to deliver five inhalations of 1 s each. Patients were instructed to take slow vital-capacity inhalations and, 10 μl of solution was administered in each breath. Before starting the BPTs, patients inhaled diluent (PBS), and variability lower than 5% between baseline and postdiluent FEV_1 values was required.

Methacholine-BPT

To avoid the circadian rhythm effect on airway dynamics, Mth-BPTs were done between 2 and 4 P.M. Methacholine

(Provocholine, Roche Laboratory. Nutley, NJ, U.S.A.) at serially increasing concentrations (0.125, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 mg/ml in PBS) was administered by the MEFAR dosimeter, and the FEV_1 value was measured by spirometry 3 min later. The test finished when a fall in FEV_1 values equal to or higher than 40% from the postdiluent value was achieved, or when the highest concentration was inhaled. Results were expressed in terms of the Mth-PD_{20} [provocative cumulative dose of Mth in μmol (1 mol Mth = 195.4 g) needed to decrease FEV_1 by 20% from the postdiluent value), and the dose–response slope (Mth-DRS): percentage of change in FEV_1 from the postdiluent values in response to the cumulative dose of Mth inhaled (24).

Allergen-BPT

Every A-BPT was done between 8 and 10 A.M. The *D. pteronyssinus* extract at increasing concentrations (0.04, 0.1, 0.2, 0.4, 1.0, 2.0 and, 4.0 ng Der p 1/ml in PBS) was administered by the MEFAR dosimeter, and FEV_1 values were recorded 10 min later. The test finished when a fall in FEV_1 values equal to or higher than 20% of the postdiluent value was achieved or when the highest concentration of allergen was inhaled. Results were expressed in terms of the A-PD_{20} [provocative cumulative dose of allergen (in ng of Der p 1] needed to decrease FEV_1 by 20% of the postdiluent values] and the dose–response slope (A-DRS) (percentage of change in FEV_1 from the postdiluent values in response to cumulative dose of allergen inhaled).

Measurements in peripheral blood

Total numbers of eosinophils were quantified in blood. Total and *D. pteronyssinus* specific serum IgE were determined with a fluoro-enzyme immunosorbent assay (UNICAP. Pharmacia Diagnostics, Uppsala, Sweden). The limits of detection for the fluid-phase assays were 2 kU/l for total IgE and 0.35 kU/l for specific IgE.

Statistical analysis

Statistical analysis of the data was performed using a statistics program (SPSS Windows 9.0; SPSS; Chicago, IL). Descriptive statistics were used to summarize the clinical and demographic characteristics of the patients. Log transformation was used to obtain normal distribution of data. Comparative analysis of means was carried out by the analysis of variance (*t*-test for equality of means). Correlation among variables was analyzed with the Pearson' rank correlation coefficient. We developed multiple models of discriminant analysis, by entering in step and in non-step fashion all the log-transformed variables. Our criterion for variable selection was the minimization of Wilks' lambda method. At each step, the variable that resulted in the smallest Wilks' lambda for the discriminant analysis was entered. The selection of variables was made according to the following criteria: Wilks' lambda: 0.623, maximum number of steps 20, minimum tolerance level 0.001, minimum F to enter 3.84, maximum F to enter 2.71. A P value of <0.05 was considered significant.

RESULTS

Tables 1 and 2 exhibit the demographic, immunological and lung-functional data by group classification. Total and specific serum IgE levels were higher in the asthma group (Table 2). All the patients underwent Mth-BPT but three subjects from the rhinitis group and six from the mild asthma group did not consent to undergo the A-BPT. The Mth-BPT results from one patient included in the AA group were not analyzed since his spirometry maneuvers were not reproducible. Mth and A-challenge were tolerated without complication and Mth- and A-PD_{20} values were obtained in every asthma patient and in 16 and 20 rhinitis patients, respectively. Groups statistically differed in PD_{20} and DRS values to both, Mth and allergen (Table 2). In the asthma group and in the whole sample, the values of Mth- and A-DRS correlated with A-PD_{20} values and, with total and specific serum IgE levels (Table 3). A degree of association was observed between Mth-DRS values and blood eosinophilia in both groups (Table 3). Only in the asthma group, the exposure to Der p 1 allergen correlated with the values of Mth-DRS and A-DRS (Table 3).

We developed multiple models of discriminant analysis by including in step or non-step fashion all the variables. The model that showed the highest discriminative power (Wilkś lambda: 0.623, $P<0.0001$) was the one that analyzed in a stepwise fashion the levels of total and specific serum IgE and the values of Mth- and A-DRS. Mth-DRS was the variable that exhibited the highest contribution to the discriminant function (Table 4). The overall percentage of cases correctly classified with this model (Table 5) was 79.2 % (61 out of the 77 patients). Mth-DRS was the first variable included in the stepwise analysis

Table 1. Patients demographic characteristics (median and interquartile range: IQR)

	AR	AA
N	24	62
Sex	16 male (66.7%)	40 male (64.5%)
Age (years)	21 (IQR: 18–23)	20 (IQR:18–29)
Duration of asthma (years)	—	2.0 (IQR: 1.0–4.25)

Abbreviations: AA: allergic asthma; AR: allergic rhinitis.

Table 2. Allergen exposure, sensitization, and lung function indices in rhinitis (AR) and asthma (AA) groups (geometric means and geometric standard deviation). Comparative analysis between groups (*t*-test for equality of means)

	AR	AA	Significance
Der p I levels (μg/g dust)	3.15 ± 6.76	1.90 ± 7.76	NS
Skin tests (mm^2)	53.70 ± 1.98	70.30 ± 1.60	NS
Blood eosinophils ($cell/mm^3$)	331.13 ± 1.79	338.84 ± 1.90	NS
Total serum IgE (kU/l)	114.80 ± 3.02	239.88 ± 2.60	$P = 0.003$
Specific serum IgE (kU/l)	17.78 ± 2.95	39.80 ± 2.09	$P < 0.001$
Baseline FEV_1 (%)	104.71 ± 1.09	100.02 ± 1.12	NS
Mth-PD_{20} (μmol)	28.84 ± 9.12	1.90 ± 7.76	$P < 0.001$
Mth-DRS (%/μmol)	0.43 ± 5.49	5.10 ± 8,43	$P < 0.001$
A-PD_{20} (ng Der p I)	14.12 ± 3.47	5.21 ± 3.55	$P = 0.003$
A-DRS (%/ng Der p I)	0.74 ± 4.46	3.43 ± 3.80	$P < 0.001$

Abbreviations: NS: not significant; Mth-PD_{20}: methacholine-PD_{20} values; Mth-DRS: methacholine dose–response slope; A-PD_{20}: allergen-PD_{20} values; A-DRS: allergen dose–response slope.

Table 3. Correlation among variables in the asthma (AA) group and in the whole sample (WS) (Pearson' rank correlation coefficient).

		Der pI	ST	t-IgE	s-IgE	Eosinop.	Mth-DRS	A-PD_{20}	A-DRS
Mth-DRS	AA	$P = 0.013$	—	$P = 0.005$	$P = 0.065$	$P = 0.038$		$P < 0.001$	$P < 0.001$
		$r = 0.46$	—	$r = 0.38$	$r = 0.25$	$r = 0.30$		$r = -0.57$	$r = 0.58$
	WS	—	—	$P < 0.001$	$P = 0.001$	$P = 0.036$		$P < 0.001$	$P < 0.001$
				$r = 0.45$	$r = 0.35$	$r = 0.36$		$r = -0.53$	$r = 0.58$
A-DRS	AA	$P = 0.037$	—	$P = 0.005$	$P = 0.045$	—	$P < 0.001$	$P < 0.001$	
		$r = 0.39$	—	$r = 0.38$	$r = 0.27$		$r = 0.58$	$r = -0.90$	
	WS	—		$P < 0.001$	$P < 0.001$	—	$P < 0.001$	$P < 0.001$	
				$r = 0.50$	$r = 0.50$		$r = 0.58$	$r = -0.91$	

Abbreviations: ST: skin tests; t-IgE: total serum IgE; s-IgE: specific serum IgE.

and, at this point, the model (Wilks' lambda: 0.672, $P < 0.0001$) correctly classified 78.8% of the cases (Table 6). These results were very similar to those obtained with the first model that also entered A-DRS and total and specific serum IgE (Table 5).

DISCUSSION

Pathogenic and epidemiological data suggest that asthma and rhinitis are linked conditions (2,7). Asthma development is associated with atopic family constitution and perennial indoor allergen exposure during the first years of life (25). Mites (*D. pteronyssinus*) are the most common indoor allergens in nearly all the world (18), but the reason why some mite-allergic patients develop asthma whereas others only present nasal symptoms is poorly understood. Several tests including exposure and sensitization to the allergen, blood eosinophils or lung-function tests with either bronchoconstricting agents or allergen

Table 4. Standardized discriminant functions coefficients and pooled within-groups correlations (structure matrix)

Variable	Standardized coefficients	Structure matrix coefficients[a]	Function[b]
Mth-DRS	0.765	0.897	1.000
A-DRS	0.298	0.728	0.467
t-IgE	−0.272	0.439	0.343
s-IgE	0.432	0.501	0.200

Abbreviations: Mth-DRS: methacholine dose–response slope; A-DRS: Allergen dose–response slope; t-IgE: total serum IgE; s-IgE: specific serum IgE.

[a] The structure matrix coefficients represent the pooled within-groups Pearson' correlation coefficients between the discriminant function and the original variables.

[b] "Function" displays the pooled within-group Pearson' correlations between discriminant variables and the typified canonic discriminant function.

Table 5. Resume table for diagnosis classification obtained with the model that entered total and specific serum IgE, Mth-DRS and A-DRS values

Observed	Predicted		Total
	AR	AA	
AR	18 (85.7%)	3 (14.3%)	21
AA	13 (23.2%)	43 (76.8%)	56
			Overall: 79.2%

This model correctly classified the 79.2% of the original cases.
Abbreviations: AR: allergic rhinitis; AA: allergic asthma.

Table 6. Resume table for diagnosis classification obtained with the model that only entered Mth-DRS values

Observed	Predicted		Total
	AR	AA	
AR	21 (87.5%)	3 (12.5%)	24
AA	15 (24.6%)	46 (75.4%)	61
			Overall: 78.8%

This model correctly classified the 78.8% of the original cases.
Abbreviations: AR: allergic rhinitis; AA: allergic asthma.

are often utilized in the evaluation of allergic asthma. In this study, mite-monosensitive patients were recruited and classified as mild asthma or rhinitis on the basis of an exhaustive clinical history that was our "gold standard" to confront groups. All the patients had been evaluated for at least 2 years and bronchial symptoms had been repeatedly discarded in the rhinitis group. We conducted several models of discriminant analysis to identify the laboratory test or combination of them that allowed the best differentiation between groups.

The absence of asthma symptoms in some patients presenting BHR has been attributed to an insufficient stimulus to cause symptomatic airway narrowing (26). Supporting such hypothesis, a moderate association between Der p 1 allergen exposure on the one hand and, sputum mast cell activation and asthma symptoms on the other was observed in AA (27). Further, higher exposure to Der p 1 was described among asthma patients displaying late pulmonary responses following A-BPTs (8). In this study and exclusively in the asthma group, Der p 1 exposure correlated with Mth- and A-DRS values. However, patients reporting asthma symptoms were not exposed to higher levels of Der p 1 allergen. Such finding is in keeping with other reports (28) and suggests that other factors (bronchial intrinsic dynamics, severity of the immunological response) should be implied in asthma presentation. As reported (29), asthma patients exhibited higher levels of total and specific IgE what suggests a more intense systemic immunological response to the allergen among subjects exhibiting asthma symptoms.

BHR is a complex functional disorder of the airways defined by dose–response curves that provide different information (30). The shift of the curve to the left indicates increased sensitivity and is mainly dependent on the loss of integrity of the respiratory epithelium (31). The severity of the response, given by a steeper DRS and either, the lack of identification of a maximal airway narrowing plateau or its detection at high degrees of airway obstruction, is presumably the most important asthma functional feature since it puts subjects at risk for serious disease (30). Bronchial response is due to both, the reduction in the forces that limit airway narrowing and the increase in airway wall thickness (31,32). All of them, inflammatory infiltrate, edema of the submucosa and deposition of collagen in the epithelial subbasement membrane can increase bronchial wall thickness and enhance the response to the agonist. Eosinophils, through the release of tumor growth factor-β_1, are likely implied in the deposition of collagen in the epithelial subbasement membrane and, a close relationship between ongoing eosinophilia and bronchial structural changes has been proposed (33). Accordingly, we observed a weak correlation among blood eosinophils and Mth-DRS values. The lack of differences in eosinophil numbers between groups might be explained since their measurement in blood might not be representative enough of the immunological response occurring at the airways (4). Although at a lesser extent than patients displaying asthma symptoms, a considerable percentage of rhinitis subjects exhibited a positive response to Mth and allergen (PD_{20} values were recorded).

Discriminant analysis mathematically obtains the best differentiation among groups of subjects using one or one set of variables (34). The model exhibiting the highest discriminative capacity (rightly diagnosed 80% of the patients) was the one that entered total and specific serum IgE, Mth-DRS and A-DRS, and confronted them to the clinical diagnosis. The lower discriminative power of both Mth-and A-PD_{20} values compared to their corresponding DRS values is attributable to the different mechanisms underlying bronchial sensitivity and response (30), as well as to the utilization of arbitrary data since PD_{20} values were not identified in every rhinitis patient. It is remarkable that the discriminative power of the model was not increased by the introduction of any other variable than Mth-DRS (entered in the first step), which strongly suggests that most of its discriminative capacity is monopolized by this variable. The higher discriminatory power of Mth-DRS values compared to the corresponding A-DRS values might be imputed to the

different FEV_1 drops induced in both challenges. Since in the Mth-challenge we induced FEV_1 drops by 40% [safe and well tolerated in asthmatics (35)], due to ethical reasons and to avoid the possibility of severe late asthmatic response (36) in the allergen challenge, only FEV_1 falls by 20% were induced.

To summarize, the major difference between mild asthma and rhinitis lies in the intrinsic behavior of the airways and, the index that best differentiates both conditions is the slope of the dose–response curve obtained with Mth. The increase in bronchial wall thickness would lead to exaggerated airway narrowing when the smooth muscle shortens and then to a steeper slope of the dose–response curve. Inflammatory changes and collagen deposits in the bronchial wall are features of asthma but have also been described in rhinitis patients who have never experienced bronchial symptoms (2,3). This finding suggests that other factors, secondary to the failure of the lung parenchyma to attenuate muscle shortening, are implied in the development of asthma symptoms (32). Due to the high prevalence of allergic rhinitis and mild asthma, we are aware of the difficulty of including complete dose–response curves to Mth in the initial evaluation of these conditions. However, given the large variability in the individual perception of airway narrowing episodes (13), their evaluation might allow a more accurate diagnosis of patients with mild bronchial symptoms who would benefit from early anti-inflammatory treatment (10). Whether such measures might prevent the development of chronic asthma should be established by prospective studies.

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